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Studies on cover crops and sudden death syndrome of soybean

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ABSTRACT

Soybean sudden death syndrome (SDS), caused by *Fusarium virguliforme,* is a major soybean disease affecting soybean production in the United States. In search for more diversified cropping systems, the adoption of cover crops in the corn-soybean rotation is being encouraged. However, there is lack of information regarding the impact that cover cropping can have on SDS. On the one hand, it is possible that the improvements in soil health caused by cover crop can create an environment that is not favorable to the disease. On the other hand, *F. virguliforme* is able to colonize many plant species and, if a host plant is chosen as a cover crop, it is possible that inoculum will increase in the soil, therefore increasing the disease.

In order to investigate this theme, two studies were conducted to determine: 1) the susceptibility of cover crop species to *F. virguliforme* and 2) the effects of rye cover crop on soybean SDS in field conditions.

In the first study, several species of cover crops were inoculated with *F. virguliforme* in the greenhouse. All legumes species tested developed root rot symptoms and showed pathogen DNA levels associated with roots similar to soybean; thus they are considered as hosts of *F. virguliforme*. In general, the *Brassica* and grass species tested did not develop typical SDS symptoms and showed minimal or no pathogen DNA associated with roots; thus they are not considered as hosts of *F. virguliforme*. The second study was conducted in a long-term field trial with two treatments: winter rye cover crop and no winter rye cover crop. The plots were artificially infested with *F. virguliforme*. In the two years of the field trial, SDS levels were low and the rye cover crop did not affect SDS development, plant growth or yield.

The findings obtained in this study are important to help us understand the relationship between SDS and cover crop. This is the first study to report crimson clover and hairy vetch as

hosts of *F. virguliforme*. In the field experiment, there was no effect of rye cover crop on SDS in subsequent soybean. Further studies are needed to investigate the effect of cover crops on SDS in field conditions.

CHAPTER 1. GENERAL INTRODUCTION

Thesis Organization

The thesis is divided into four chapters. The first chapter includes the literature review of the history, distribution, symptomatology, and management of sudden death syndrome (SDS), as well as the justification of the research conducted. The second chapter is a study on the cover crop host range of *Fusarium virguliforme* and *Heterodera glycines*. The third chapter describes a field study on the effect of a rye cover crop on SDS, and the fourth chapter provides a summary and the general conclusions of the thesis.

General Introduction

In the United States, SDS is caused by *Fusarium virguliforme* Aoki, O'Donnell (Aoki et al. 2003). This soilborne fungus infect the roots of soybean causing root rot and also releases a phytotoxin which causes foliar chlorosis and necrosis (Roy et al. 1997). SDS was considered the fourth most important soybean disease in the United States during 2006 to 2009 (Koenning and Wrather, 2010). According to Wrather and Koenning (2009), losses due to SDS averaged $190 million a year from 1996 to 2007.

Management of SDS is considered difficult. One of the limitations in controlling the disease is lack of understanding of the ecology of the pathogen and factors that favor pathogen development (Xing and Westphal, 2006). Currently, the preferred and most cost-effective method to control SDS consists of using resistant cultivars in combination with fungicide seed treatment (Kandel et al. 2016). Other cultural practices such as crop rotation have been reported to reduce disease, although results have been inconsistent. Rupe et al. (1997), for example, found lower *F. virguliforme* densities in soil from fields with a sorghum-wheat rotation compared to a fescue-soybean rotation. On the other hand, the two-year corn-soybean rotation sequence that is predominant in the Midwest region has been shown to be highly favorable to SDS (Xing and Westphal, 2009). In a recent study, Navi and Yang (2016) reported that corn residue can harbor *F. virguliforme* in the absence of soybean. Moreover, Abdelsamad et al. (2012) found higher SDS incidence and severity in a two-year corn-soybean rotation compared to a three-year corn-soybean-oat/red clover rotation, and four-year corn-soybean-oat/alfalfa-alfalfa rotation. These results suggest that longer crop rotation sequences might be a good management practice for reducing the risk of SDS.

In recent years, interest in cover crop as a cultural practice has increased among farmers because of their benefits to water and soil quality (Carlisle, 2016; Dabney et al. 2001). However, there is lack of information on the impact that cover crops may have on plant diseases, and specifically on SDS. It is possible that an increase in *F. virguliforme* inoculum in the field can occur if a given cover crop is a host to the pathogen, allowing the fungus to survive and reproduce in the absence of its primary host. Based on the literature, the host range of *F. virguliforme* consists of mostly leguminous species and a few plants from other families (Kolander et al. 2012; Melgar and Roy, 1994). Hence, it is essential to determine which cover crops species are hosts or non-hosts to *F. virguliforme* to better understand how they can affect the pathogen. In the research presented in this thesis, a greenhouse experiment was conducted to test the potential of several cover crop species to host *F. virguliforme*.

Rye (*Secale cereale*) has been the predominant cover crop planted in the Upper Midwest United States (De Bruin et al. 2005), probably because of the ability of rye to survive low temperatures in the winter. However, there are no conclusive studies of the impacts of rye cover crop plantings on SDS. In the research presented in this thesis, a long-term rye cover crop field trial was inoculated with *F. virguliforme* to investigate this subject.

Therefore, two objectives will be addressed in the research presented here: 1) the susceptibility of cover crop species to *F. virguliforme* infection and 2) the effect of rye cover cropping on soybean SDS in field conditions.

Literature Review

Soybean

Soybean (*Glycine max*) is one of the most important legumes grown in the world. In the United States, soybean was grown on more than 33 million hectares in 2016 (USDA/NASS), with more than 80% of production concentrated in the Midwest. The corn and soybean rotation is grown on more than 20 million hectares in the North Central region in the United States (Chen et al. 2006). Unfortunately, many factors can negatively affect soybean yield, such as insect pests, nematodes and soilborne diseases. From 2001 and 2003, the estimated worldwide yield loss due to soybean diseases was 23% (Oerke, 2006).

History and distribution of soybean sudden death syndrome

SDS was first observed in Arkansas in 1971 (Roy et al. 1997) and since then has quickly spread throughout most of the soybean growing regions. SDS has been reported in Mississippi, Missouri, Kentucky and Tennessee (Rupe, 1989), Illinois (Hartman et al. 1995), Kansas (Jardine and Rupe, 1993), Minnesota (Kurle et al. 2003), Kentucky (Hershman et al. 1990), Michigan (Chilvers and Brown-Rytelewski, 2010), Nebraska (Ziems et al. 2006), Iowa (Yang and Rizvi, 1994), Ontario (Anderson and Tenuta, 1998), Wisconsin (Bernstein et al. 2007), South Dakota (Tande et al. 2014), and Louisiana (Singh et al. 2015). In South America, the disease has been reported in Brazil (Arruda et al. 2005) and Argentina (Scandiani et al.

2004). More recently, SDS was detected for the first time in South Africa (Tewoldemedhin et al. 2016).

Causal agent of SDS

To date, there are four *Fusarium* species known to cause SDS on soybeans. In North America, *F. virguliforme* is the sole species responsible for causing SDS (Aoki et al. 2005). This species was formerly known as *F. solani*, later as *F. solani* f. sp. g*lycines* (Roy, 1997) and renamed by Aoki et al. in 2005. In South America, three additional Fusarium species are recognized for causing SDS: *F. brasiliense*, *F. tucumaniae* and *F. crassistipitatum* (Aoki et al. 2012).

Symptomatology

Early in the soybean growing season, SDS development is favored by cool and wet soil conditions, and later in the season, by high soil moisture (Scherm and Yang, 1996). SDS is known to cause symptoms on soybean roots and foliage (Roy et al. 1997). The root symptoms are characterized by root necrosis of the tap and lateral roots. During root colonization, the fungus releases a toxin that is translocated through the vascular system to the aboveground plant tissue, and this toxin uptake will cause the typical foliar symptoms (Brar et al. 2011). Foliar symptoms start as yellow, circular mottling on leaves and will eventually advance to interveinal chlorosis and necrosis (Navi and Yang, 2008). In high infestations, leaflets will prematurely drop off from the plant, leaving the petioles attached to the plant. Early defoliation is responsible for most yield damage. Foliar symptoms rarely appear before reproductive stages (Hartman et al. 2015).

Host range

Even though soybean is considered to be the primary host of *F. virguliforme*, the SDS pathogen has a broader host range (Gray et al. 1999). Mung bean (*Vigna radiate*) and green bean (*Phaseolus vulgaris*) develop root rot and foliar symptoms, following inoculation without wounding. When inoculated after wounding, lima bean (*P. lunatus*) and cowpea (*V. unguiculata*) also developed symptoms, thus being considered as hosts (Melgar and Roy, 1994). Kolander et al. (2012) tested the host range of fifteen different crops, prairie plants and weeds species in the greenhouse and found alfalfa (*Medicago sativa*), pinto and navy beans (*P. vulgaris*), white clover (*Trifolium repens*) and red clover (*T. pretense*), pea (*Pisum sativum*), Canadian milk vetch (*Astragalus canadensis*), sugar beet (*Beta vulgaris*) and canola (*Brassica napus*) were symptomatic hosts of *F. virguliforme*. Corn (*Zea mays*), wheat (*Triticum aestivum*), ryegrass (*Lolium multiflorum*), pigweed (*Amaranthus retroflexus*), and lambsquarters (*Chenopodium album*) did not develop symptoms but had similar amounts of pathogen DNA in the inoculated roots compared to soybean, suggesting that they were asymptomatic hosts.

Management strategies

Managing SDS is very challenging because there is not a single management practice that is completely effective at reducing the disease (Leandro et al. 2013). Research has shown that planting soybean varieties with some level of resistance to SDS is an effective tool to reduce disease risk (Leandro et al. 2013). However, it is important to note that resistance alone may not provide full control of SDS in highly conducive years (Kandel et al. 2016). Current breeding programs have been releasing new varieties with partial resistance to SDS (Cianzio et al. 2014). The use of fungicide seed treatments for managing SDS has only recently shown positive results in the field. Kandel et al. (2016) tested 16 different fungicides treatments, but only fluopyram as a seed treatment and as an in-furrow application showed good results, with up to 95% reduction in SDS severity. Currently, the primary options for disease control are the use of resistant cultivars and seed treatment with fluopyram (Kandel et al. 2016).

Some studies have shown that delayed planting may reduce SDS severity (Hershman, et al. 1990; Wrather et al. 1995). According to Hershman et al. (1990), this response could be because the fungus benefits from the cool and wet soil conditions present when soybean is planted early in the growing season. However, in the Midwest, delayed plating is not recommended as a management practice for SDS because it can be more detrimental to yield than the disease (DeBruin and Pedersen, 2008; Kandel et al. 2016; Marburger et al. 2016). In fact, Kandel et al. (2016) found that early (late April to early May) soybean planting is not always correlated with higher levels of SDS.

The effect of tillage on SDS is not very well understood (Westphal, 2011). Some studies have shown that soil tillage reduces the severity of SDS compared to no-till soils (Vick et al. 2003; Wrather et al. 1995). One possible reason might be because tilled plots, in general, have lower soil bulk density, greater soil porosity, and less soil moisture (Chong et al. 2005; Scherm et al. 1998), which are soil conditions that do not favor SDS.

Crop rotation can be a useful control management for soilborne diseases (McMullen and Lamey, 1999). Disease reduction can be achieved by reducing pathogen population in the soil or increasing populations of antagonistic organisms (Krupinsky et al. 2002). On SDS, however, rotation with some crops may not be as effective (Rupe et al. 1997). Some studies have shown that corn in rotation with soybean actually favors the disease (Navi and Yang, 2016; Xing and Westphal, 2009). On the other hand, soybean in rotation with sorghum or wheat decreased inoculum densities of *F. virguliforme* compared to continuous soybean (Rupe et al. 1997). Knowledge of the host range is key for successful management (Krupinsky et al. 2002).

The interaction between the soybean cyst nematode (SCN, *Heterodera glycines*) and SDS has been extensively studied, but the relationship between them is not clearly understood (Marburger et al. 2013; McLean and Lawrence, 1993; Melgar et al. 1994). Some studies have shown greater incidence and severity of SDS when *H. glycines* is present (McLean and Lawrence, 1993; Xing and Westphal, 2006). In contrast, Gao et al. (2006) did not find a positive correlation between *H. glycines* and SDS foliar symptoms in a greenhouse experiment. In fact, they observed a reduction of *H. glycines* reproduction per root in plants highly infested with *F. virguliforme.*

Cover crops

A cover crop can be defined as a non-harvested crop grown in the fallow season to cover the ground, protect the soil from erosion, and improve soil health (Carlisle, 2016; Reeves, 1994). Cover crops can provide multiple soil, agricultural production, and environmental benefits (Blanco-Canqui et al. 2015; Power and Biederbeck, 1991). Cover crops have proven to alleviate soil compaction (Williams and Weil, 2004), improve soil structural properties (Snapp et al. 2005), decreases soil water evaporation (Munawar et al. 1990), improve microbial community (Abawi and Widmer, 2000), recycle nutrients (Kaspar et al. 2012) and suppress weeds and pathogens (Mischler et al. 2010; Larkin et al. 2012). The adoption of cover crops has been encouraged in the Midwest to meet the need for improved soil and water conservation in fields planted with a corn-soybean rotation (Kladivko et al. 2014). Chen et al. (2006) mentioned that cover crop planting is considered an efficient practice to increase diversity in cropping systems.

In general, most practices that maintain or improve soil health also have a positive effect on reducing soilborne diseases (Larkin, 2015). That is because soilborne diseases tend to be more damaging when soil conditions are poor, e.g., in soils with low organic matter, poor drainage, poor soil structure, and high soil compaction (Abawi and Widmer, 2000). Cover cropping has provided promising results controlling soilborne diseases. For example, soil population and incidence of *Thielaviopis basicola* in cotton seedlings were reduced following a hairy vetch cover crop compared to winter fallow (Rothrock et al. 1995). Moreover, growing *Brassica juncea* as a cover crop decreased the incidence of sugar beet root rot caused by *Rhizoctonia solani* (Motisi et al. 2009).

For SDS, however, there are not many studies investigating the effect of cover crops. In a field experiment, Wen et al. (2012) tested the suppression of soybean diseases through the use of rye, rape (*B. napus*), canola (*B. napus*) and mustard (*B. juncea*) cover crops and did not observe significant differences in SDS levels in cover crop treatments in one year. According to Abawi and Widmer (2000), every crop production practice has a direct or indirect impact on root disease because cultural practices can influence the population densities of soilborne pathogens and also be beneficial or detrimental for other soil microflora and fauna. Eastburn (2014) observed some levels of reduction on SDS in rye plots soils in some years and locations, but the effect was not consistent. Therefore, cover crops should continue to be investigated as a potential tool for reducing the risk of SDS.

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CHAPTER 2. SUSCEPTIBILITY OF COVER CROP PLANTS TO *FUSARIUM VIRGULIFORME*, CAUSAL AGENT OF SOYBEAN SUDDEN DEATH SYNDROME, AND *HETERODERA GLYCINES*, THE SOYBEAN CYST NEMATODE

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(work presented on SDS was conducted by Renan Kobayashi)

Abstract

Greenhouse studies were conducted to evaluate the susceptibility of cover crop species to infection by *Fusarium virguliforme*, a soilborne fungus that causes sudden death syndrome (SDS) of soybean, and the soybean cyst nematode (SCN, *Heterodera glycines*), an important soybean pathogen. In the SDS experiments, cover crops were planted in steam-pasteurized soil amended with *F. virguliforme*-infested sorghum and grown under greenhouse conditions. The plants were assessed for fresh biomass, root rot severity, foliar symptoms, and amount of *F. virguliforme* DNA in roots. In the SCN experiment, select leguminous and non-leguminous cover crop plants were grown in soil naturally infested with SCN, and the number of females formed per root after 30 days was determined. In the SDS experiment, inoculated alfalfa, crimson clover, red clover and pea had more root necrosis than the non-inoculated controls ($P < 0.05$), and *F. virguliforme* DNA quantities in inoculated roots did not differ from those found in inoculated soybean roots ($P > 0.05$). Inoculated alfalfa, corn, crimson clover, oat, red clover, sorghum and turnip plants had lower biomass compared to non-inoculated controls ($P < 0.05$), although corn, oat and turnip had no root necrosis. Biomass reduction and root necrosis were not

observed in inoculated hairy vetch, false flax, millet, mustard, cereal rye, ryegrass, triticale and wheat, and *F. virguliforme* DNA quantity in the roots of these species was lower ($P < 0.05$) than in soybean. Because grass and *Brassica* spp. crops did not develop root rot and had small amounts of *F. virguliforme* DNA in root tissue, they may be minor contributors to inoculum buildup in soil and preferred cover crop choices for fields with a history of SDS. There were very few (zero to five) SCN females formed on the roots of multiple varieties of leguminous cover crop species studied in the experiment. No females were recovered from the roots of any of the non-leguminous cover crop species studied in the experiment except for a single female on four plants from three different species. None of the cover crop plants studied were susceptible hosts for SCN.

Key words: cover crops—soybean—sudden death syndrome—soybean cyst nematode

Introduction

Soybean (*Glycine max* [L.] Merr) is a legume species considered one of the most important economic crops grown in the world (Rosa et al. 2015). However, its development is subject to biotic stresses such as pathogens, insects and weeds that can negatively affect yield. Two major pathogens causing yield losses in soybeans are *Fusarium virguliforme* O'Donnell & T. Aoki, the causal agent of soybean sudden death syndrome (SDS) (Aoki et al. 2005), and *Heterodera glycines* Ichinohe, the soybean cyst nematode (SCN) (Ichinohe, 1952).

SDS affects soybean production in the United States, especially in the Midwest where it is considered one of the most important soybean diseases due to its potential to decrease yield (Hartman et al. 2015; Koenning and Wrather, 2010). Symptoms of SDS include root rot and interveinal chlorosis and necrosis on leaves (Rupe, 1989). The foliar symptoms are caused by a phytotoxin (Brar et al. 2011) that is translocated from the roots to the aerial parts of the plant.

Management options to control SDS, such as plant resistance (Cianzio et al. 2014; Hartman et al. 2015; Mueller et al. 2003), crop rotation (Abdelsamad et al. 2012; Rupe et al. 1997) and seed treatment (Kandel et al. 2016), are limited and not always effective (Hartman et al. 2015; Swoboda et al. 2011; Xing and Westphal, 2006).

It is widely considered that SCN is the most destructive pest of soybean in the United States (Bradley et al. 2014; Wrather and Koenning, 2009). The pathogen can be managed by growing SCN-resistant cultivars and/or non-host crops (Chen et al. 2001; Donald et al. 2007) and by using nematode-protectant seed treatments. Chen et al. (2006) suggested that effective management of SCN populations in soil in the Midwest requires the adoption of a more diversified cropping system than the corn (*Zea mays* L.)-soybean rotation that is prevalent in the region and that cover crops may contribute to increasing cropping system diversity.

The interaction of *F. virguliforme* and SCN results in more severe SDS than when soybeans are infected with *F. virguliforme* alone (McLean and Lawrence, 1993; Melgar and Roy, 1994; Xing and Westphal, 2006). The fact that these pathogens commonly occur together in fields (Gao et al. 2006; McLean and Lawrence, 1995) makes their interaction particularly important when considering soybean disease management practices.

The predominant row crops production system in the Midwestern United States is based on 2-year corn-soybean rotation (Chen et al. 2006). Recent nutrient reduction strategies have been implemented in this region (King et al. 2016; Porter et al. 2015) to reduce nutrient loss from this corn-soybean system. Planting cover crops have been suggested as part of the solution (Arbuckle and Roesch-McNally, 2015; Dabney et al. 2001; Sarrantonio and Gallandt, 2003) because this practice is known to improve soil quality, reduce erosion and improve weed control (Drury et al. 2014; Kaspar and Singer, 2011; Larkin et al. 2010; Snapp et al. 2005). However, the impact that cover crops may have on soilborne diseases is unknown (Rupe et al. 1997). It is possible that some cover crop species can be hosts to particular pathogens and increase inoculum density in the field, or that cover crops can affect the microenvironment and reduce inoculum density.

The host range of *F. virguliforme* includes several leguminous species, as well as plants in other families. Kolander et al. (2012), for example, inoculated fifteen different crops, weeds and prairie plants with *F. virguliforme* in the greenhouse and found that several of the legumes developed root rot. Early host range work done by Melgar et al. (1994) found that edible green bean, lima bean and cowpea became infected after wound inoculation. However, little is known about cover crops that could serve as hosts for *F. virguliforme*. The host range of SCN is considered broad (Turner and Subbotin, 2013), although plant species vary in their ability to serve as a host. Plant species from Fabaceae family are considered the main host plant family (Turner and Subbotin, 2013), but *H. glycines* can also reproduce on plants from other families (Riggs, 1987). To date no published study has focused on determining the status of cover crops as hosts of SCN.

To better understand how cover crops can affect soybean diseases such as SCN and SDS, it is important to first understand which cover crop species can be hosts to these pathogens. The objective of this study was to determine the host range of *F. virguliforme* and *H. glycines* on different cover crop species.

Material and Methods

SDS experiment

The experiment was conducted in a greenhouse on the Iowa State University campus in Ames, Iowa. Fifteen cover crops species belonging to 14 different genera were selected for this study (table 1). Two soybean cultivars, and one corn hybrid, were used as controls. The soybean variety Kruger K287RR is ranked as highly resistant to SDS and the variety K2918 is ranked as susceptible.

The *F. virguliforme* isolate NE 305 used in this study was recovered in 2006 from roots of a SDS symptomatic plant in Nevada, IA. The isolate was grown on Potato dextrose agar at 23ºC (73ºF), for 12 days, in the dark, and was used to produce inoculum by infesting commercial sorghum grain (Mueller et al. 2002). Sorghum was soaked in tap water for 24 hours, drained of excess water, then placed in mesh bags and autoclaved twice for 40 minutes on two consecutive days. Ten agar plugs of the *F. virguliforme* isolate were added to the bags, which were then incubated for 4 weeks in the dark at 23ºC (73ºF), mixing daily to provide uniform growth of the colonies in the grain. The inoculum then was dried in an airflow hood for 48 hours. This procedure was repeated for the non-inoculated control by adding non-infested agar plugs to the sorghum.

Seeds of each plant species were planted in 12.7 cm (5 inch) plastic pots containing infested or non-infested soil. Steam-pasteurized soil and sand (1:1 vol:vol) was thoroughly mixed (1:30 vol:vol) with infested or non-infested sorghum (Luckew, 2012). Seeds were surface disinfested by immersing in 10% sodium hypochlorite for 1 min, followed by rinsing 1 min in sterile distilled water. Planting density was adjusted according to seed size, and plants were later thinned to a minimum number per plants/pot (table 1). Plants were maintained in the greenhouse for 5-6 weeks, at 22ºC ±5 (72ºF), and with a 14 h photoperiod. Fertilizer (Osmocote 14-14-14) was added to the pots 7 days after planting. Plants were watered as needed to maintain adequate soil moisture. The experiment was set in a randomized complete block design in a factorial arrangement of two treatments: Crop (16 species plus 2 soybean varieties) and inoculation (inoculated and non-inoculated with *F. virguliforme*). Each pot represented an experimental unit and was randomly assigned within each block. The experiment was conducted three times, and there were 10 blocks in run 1, and 5 blocks in runs 2 and 3.

Plants were removed from the pots for disease assessments. Shoots and roots were separated from the plants at the soil line using a scissor and carefully washed in running tap water to remove soil. Foliar disease severity, root rot severity, fresh weight and dry weight were assessed for each plant. Root rot severity was estimated visually as the percentage of root area showing brown discoloration. Shoots and roots were weighed separately before drying to determine plant biomass, and after oven-drying at 60ºC (140ºF) for 24 hours to obtain dry weights.

Dry root tissue obtained from each pot was combined into one sample, ground using Thomas Wiley Mini-mill (3383-L40, Thomas Scientific, Swedesboro, NJ), and stored in vials at room temperature. Each vial contained the root tissue from one pot of plants. For DNA extraction, three pots were randomly selected from the inoculated treatments and one pot was randomly selected for the non-inoculated treatment. DNeasy Plant Mini Kit (Qiagen, Germantown, MD) was used for DNA extraction. Approximately 50 milligrams of root tissue were used for DNA extraction per sample. Due to limited amount of root tissue from crops such as red clover, false-flax and mustard, some inoculated treatments had to have replications combined in order to obtain enough tissue for extraction. The quantity of *F. virguliforme* DNA present in the roots was determined by the quantitative PCR (qPCR) assay developed by Chilvers-Wang (Kandel et al. 2015; Wang et al. 2015).

SCN experiments

The experiment was conducted in the same greenhouse as the *F. virguliforme* experiment described above. Nine leguminous cover crops species, representing five genera, were included in one set of experiments (table 2) and eight non-leguminous cover crop plants representing five species were included in another experiment (table 3). The susceptible soybean variety Williams 82 was included in every experiment as a positive control. Cover Crop Solutions, LLC, provided the seeds of the leguminous cover crop plants used in the experiment. There were multiple varieties of alfalfa, hairy vetch, red clover and white clover included in the experiments for which variety names were not available. Three seeds of the cover crop plants or soybean were sown directly into plastic cone-tainers (Stuewe & Sons, Inc., Tangent, OR) filled with soil that was infested with SCN. Initially, cone-tainers were observed every day, and the second and third seedlings that emerged within each cone-tainer were removed and discarded. Consequently, only the first seedling that emerged in each cone-tainer was used in the experiment. Cone-tainers were then placed into randomly determined positions in 19-liter-capacity plastic buckets that were filled with sand to distribute temperature for all of the cone-tainers. The buckets were placed into water baths maintained at 27˚C (80.6˚F) for 30 days, which allowed enough time for adult females to form on the roots (Niblack et al. 2009). Each bucket was placed under natural and supplemented lighting conditions and plants were watered as needed.

Soil from two different fields was used for two experiments with the leguminous cover crop species. This was done to determine if soil texture, SCN population and/or other edaphic factors would have an effect on the results. Soil in the first experiment was from Muscatine County, IA, and was infested with an HG Type 2.5.7 SCN population (Niblack et al. 2002). The nematode-infested soil was mixed with sand to obtain an initial population density of 1,900 eggs per 100 cm^3 soil for the experiment. The soil used in the second run of the experiment was from Webster County, IA, and was infested with an HG Type of 2.5.7 SCN population at an initial density of 2,500 eggs per 100 cm^3 soil. Leguminous species experiments were conducted in two runs using the Muscatine County soil with 6 replications per treatment, and three runs using the Webster County soil containing 5 or 6 replications per treatment. Non-leguminous species experiments were carried out in two runs with soil from Muscatine County infested with SCN HG type 2.5.7, only. All other details were the same as for experiments with the leguminous cover crop species.

In each run of each experiment, the soil and roots from each individual cone-tainer were carefully removed after 30 days of incubation, then the soil from each root system was carefully washed away. Roots were subsequently placed on a sieve with 850-µm-diameter pores nested over a sieve with 250-µm-diameter pores and sprayed with a strong stream of water. The stream of water dislodged the SCN females from the roots. The SCN females passed through the top, 850-µm-pore sieve and were collected on the bottom, 250-µm-pore sieve. The SCN females collected on the bottom sieve and all other debris on that sieve were observed with a dissecting microscope, and the number of SCN females recovered from each individual plant were counted.

Statistical analysis

For the SDS experiment, the PROC MIXED procedure (SAS version 9.4, SAS Institute Inc., Cary, NC) was used to test for significant differences between *F. virguliforme*-inoculated and non-inoculated treatments within each plant species. Experimental run was a random factor in the model; runs were combined in the final analysis as the run x inoculation interaction was insignificant in most data sets. Differences in mean root rot severity and fresh plant weight for inoculated and non-inoculated treatments were tested at the $P = 0.05$ level. The quantity of DNA in roots samples was reported as picograms of *F. virguliforme* DNA/ 50 ng of total DNA. DNA quantities of individual crop species were compared to the mean quantity in the soybean samples ($P = 0.05$).

For the SCN experiments with the leguminous cover crops, numbers of SCN females per root were transformed for analysis (log10 for Webster County soil, ln for Muscatine County soil). The SCN female data were log10 transformed for the experiment with the non-leguminous cover crop species. Data were subjected to ANOVA using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC) followed by contrast statements of susceptible soybeans versus cover crops and then pairwise comparisons among cover crop species.

Results and Discussion

SDS Experiment

Foliar symptoms were minimal on soybean and not observed in the other plant species. In general, legumes species inoculated with *F. virguliforme* developed more root rot ($P < 0.05$) than the non-inoculated treatments. Root rot was observed on soybean and several other plant species grown in infested soil, and ranged from 0 to 87% (Fig. 1). Soybean, alfalfa, crimson clover, pea, red clover and hairy-vetch consistently developed significant ($P < 0.05$) root rot when compared to non-inoculated treatments. *Brassica* spp did not develop significant root rot in any experimental run. The same trend was also observed in most of the grasses species tested, but sorghum, triticale and wheat developed root rot in at least one experimental run. The root rot results are consistent with previously reported by Kolander et al. (2012), who also found that inoculated alfalfa, pea and red clover developed significant root rot. This is the first study reporting hairy-vetch and crimson clover as hosts of *F. virguliforme*.

Inoculated soybean, alfalfa, crimson clover and red clover showed significant biomass reduction when compared to non-inoculated plants (figure 2). Hairy-vetch and pea did not show biomass reduction even though significant root rot was observed. No significant difference was observed in biomass for false-flax, mustard, turnip, millet, oat, ryegrass, and rye (figure 2).

F. virguliforme is has been shown to reduce biomass on soybean (Gao et al. 2006; Gongora-Canul et al. 2012; Luckew et al. 2012). In this study, the inoculated soybean plants had 20% less biomass compared to the non-inoculated treatment (figure 2B). Gongora-Canul et al. 2012 found reduction up 67% on root biomass on plants inoculated by *F. virguliforme*. Luckew et al. (2012) results also showed significant loss in root dry weight on a susceptible genotype compared to a resistant genotype. Kolander et al. 2012, reported reduction between 50 and 60% on soybean biomass.

In run 1 (figure 3A), qPCR results detected on soybean roots ranged from 53 to 210 pg *F. virguliforme* DNA/10ng of DNA, averaging 121 pg. The amount of *F. virguliforme* DNA detected in the inoculated roots of alfalfa, red clover, crimson clover, hairy-vetch and pea did not differ statistically from soybean. On the other hand, quantity of *F. virguliforme* in the roots of inoculated false-flax, mustard, turnip, corn, millet, oat, ryegrass, rye, sorghum, triticale, and wheat (figure 3A) was lower than in inoculated soybean. Results for qPCR from run 2 were not reported here due to poor quality of DNA extracted from roots. In run 3 (figure 3B), the amount of *F. virguliforme* DNA detected in the inoculated soybean ranged between 0.15 to 291 pg/10 ng of DNA, and did not differ significantly among the crops tested, except for crimson clover that showed greater *F. virguliforme* DNA than soybean. The DNA analysis results of this study contrast with Kolander et al. (2012), where similar amounts of pathogen DNA were detected in the roots of inoculated corn, wheat and ryegrass compared to inoculated soybeans, even though foliar symptoms and biomass reduction was not observed.

The data collected in this study suggests that the legume species tested (alfalfa, crimson clover, hairy-vetch, pea and red clover) are hosts of *F. virguliforme* because they consistently developed root rot and had DNA levels in roots similar to soybean, indicating pathogen infection. However, the results obtained for false-flax, mustard, turnip, corn, millet, oat, ryegrass, rye, sorghum, triticale, and wheat, do not provide enough evidence to conclude that the roots are colonized by *F. virguliforme*, suggesting that these crops are non-hosts or poor hosts.

SCN Experiments

In some instances, there were less than six live plants remaining at the end of a run of an experiment (tables 2 and 3). Therefore, the number of replications varied among the different cover crop plants.

Very few SCN females formed on roots of most of the leguminous cover crop plants included in our experiments. There was zero to three SCN females per root on alfalfa, berseem clover, crimson clover, red clover and white clover plants. There were zero to 15 females on the roots of individual pea and cowpea plants, but about one third of the individual pea and cowpea plants had no SCN females on the roots. There was an average of 72 to 256 SCN females per root on the susceptible soybean in the various runs of the legume experiments, indicating that conditions were conducive for infection and reproduction of the nematode during the experiments.

The number of SCN females found on the susceptible soybean control was significantly greater (P = <0.001) than all leguminous cover crop species in the experiments (table 2). And although numbers of SCN females produced on all of the cover crops was very low, there were significantly ($P < 0.05$) more females per root on peas compared to both alfalfa species and red clover-1 in both soils. Also, one hairy vetch cultivar (3) had significantly ($P < 0.05$) more SCN females per root compared to the two alfalfa species in the experiments with the Webster County and Muscatine County soils, Austrian winter pea, hairy vetch-1 and crimson clover had significantly ($P < 0.05$) more females per root compared to alfalfa-1 and alfalfa-2 in the experiment with SCN-infested soil from Webster County. None of these significant differences among the cover crops will have any practical significance, however, and the numbers were low overall.

The numbers of SCN females per root of non-leguminous cover crops were significantly lower ($P < 0.001$) than those found on the susceptible soybeans, and there were no significant differences ($P > 0.05$) in number of females per root of non-leguminous cover crop species (table 3).

The results of the three different greenhouse experiments with 2 to 3 runs each revealed that there was very little reproduction of SCN on all of the leguminous and non-leguminous cover crop plants studied. This research indicates that the cover crops studied in our experiments are not hosts of SCN and farmers need not be concerned about inadvertently providing a host that would increases SCN population densities if these plants were grown as cover crops in SCN-infested fields.

Summary and Conclusions

The findings of our greenhouse studies raise questions about the potential impact of using cover crop species in fields with a history of SDS. It would be interesting to determine if host species can increase risk of SDS, and if non-host species can suppress SDS, in field conditions. According to Levenfors (2003), the inoculum density of a pathogen decreases in soil in the absence of a suitable host plant, and it increases in the presence of a host. Other studies have shown that using rye and ryegrass as cover crops in conjunction with an effective rotation crop in the summer decreased black scurf and common scab on potato (Larkin et al. 2010). A recent study (Eastburn 2014) also observed less SDS in soybeans grown after a rye cover crop than in soybeans without rye cover crop preceding planting. It is important to note that cover crops may also reduce pathogen population in the soil by promoting the population of antagonistic microorganisms by promoting soil health (Larkin 2015).

Although this study focuses on the ability of *F. virguliforme* to infect live plants, it is known that *F. virguliforme* is able to survive and reproduce on plant residue of non-host (Navi and Yang, 2016). Therefore, it is possible that the residue derived from cover crops can support growth of *F. virguliforme* and serve as inoculum between seasons. Further studies are needed to determine if asymptomatic hosts can increase *F. virguliforme* inoculum in the soil and if the residue derived from non-hosts and asymptomatic hosts them may or may not play a role in the pathogen survival.

The results of our greenhouse experiments with SCN revealed that the cover crop plants that we examined in our experiments were not good hosts for SCN and, consequently would not result in inadvertent increases in SCN population densities if used as cover crops in SCN-infested fields. However, the experiments do not provide information supporting or refuting claims that some cover crop species reduce population densities of SCN when grown in fields infested with the nematode. Additional greenhouse and field research is underway to determine if suppressive effects are true and, if so, under what conditions.

Increasing knowledge about the role cover crops relative to common soybean pathogens will possibly help farmers select the best cover crop species for their field. Future work is needed to determine the impact under field conditions.

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Table 1

Plant species and number of plants per experimental unit (pot) evaluated as hosts of *Fusarium virguliforme*.

Plant Species	**Scientific Name**	**Variety**	**Number of seeds planted/pot**	**Number of plants/pot after thinning**
Soybean	*Glycine max*	Kruger 287 RR Kruger 2918	6	4
Corn	*Zea mays*	NA*	3	2
Oat	*Avena sativa*	IN09201	12	10
Alfalfa	*Medicago sativa*	Pioneer 53h92	15	12
Pea	*Pisum sativum*	NA*	6	4
Hairy Vetch	*Vicia vilosa*	NA*	12	6
Red clover	*Trifolium pratense*	Duration	15	12
Crimson clover	*Trofolium incarnatum*	Dixie	15	12
Mustard	*Brassica juncea*	Ames 26161	15	7
Turnip	*Brassica rapa*	Purple Top	15	7
Ryegrass	*Lolium multiflorum*	WinterHawk	15	15
Wheat	*Triticum aestivum*	Sturdy	12	10
Triticale	*Triticale hexaploide*	NA*	12	10
False flax	*Camelina sativa*	Yellowstone	15	10
Winter Rye	*Secale cereale*	Spooner	12	10
Pearl Millet	*Pannisetum glaucum*	NA*	12	7
Sorghum	*Sorghum bicolor*	BMR	12	10

*NA = not available

Table 2

Number of soybean cyst nematode (SCN) females per root produced on leguminous cover crop species and susceptible soybean, Williams 82, in soil naturally infested with SCN from Webster County and Muscatine County, Iowa, USA.

		Webster County soil		Muscatine County soil	
common name	**scientific name**	**number of SCN females per root***	**number of plants†**	**number of SCN females per root***	**number of plants†**
alfalfa - 1‡	*Medicago sativa*	0.20	15	0.20	5
alfalfa - 2	*Medicago sativa*	0.00	14	0.20	5
Austrian winter pea	*Pisum sativum* subsp. *arvense*	1.88	16	1.20	5
berseem clover	*Trifolium alexandrinum*	0.86	7	-	-
cowpea	*Vigna unguiculata*	1.15	13	-	-
crimson clover	*Trifolium incarnatum*	3.40	15	0.17	6
hairy vetch - 1	*Vicia villosa*	1.40	10	0.33	6
hairy vetch - 2	*Vicia villosa*	1.10	10	0.86	7
hairy vetch - 3	*Vicia villosa*	1.83	12	1.73	11
peas	*Pisum sativum*	2.11	18	4.27	11
red clover - 1	*Trifolium pretense*	0.90	10	1.40	5
red clover - 2	*Trifolium pretense*	0.38	5	-	-
red clover - 3	*Trifolium pretense*	0.10	10	-	-
white clover - 1	*Trifolium repens*	0.40	5	-	-
white clover - 2	*Trifolium repens*	0.00	2	-	-
soybean, SCN susceptible	*Glycine max*	220.17	18	112.50	12

* Data were transformed for analysis (log10 for Webster Co. soil, ln for Muscatine Co. soil), but untransformed means are presented. Numbers of SCN females on Williams 82 were greater ($P<0.05$) than on all cover crop plants, and there were a few significant pairwise differences in number of females between cover crops (see text).

† There were three runs of the experiment using Webster County soil with five or six replicates per run for a total of 18 possible plants and two runs of the experiment using Muscatine County soil with six replicates per run. Not all plants survived to the end of the experiment. Data for species for which there was less than 5 replications total are not presented and were not included in the analyses.

‡ Cultivar names were not available; multiple cultivars of the same species were assigned numbers.

Table 3

Number of soybean cyst nematode (SCN) females per root produced on non-leguminous cover crop species and susceptible soybean in soil naturally infested with SCN from Muscatine County, Iowa, USA.

common name	**scientific name**	**cultivar name**	**number of SCN females per root***	**number of plants†**
annual ryegrass	*Lolium multiflorum*	King	0.09	11‡
annual ryegrass	*Lolium multiflorum*	Tillage RootMax	0.00	10
canola	*Brassica napus*	Baldur	0.00	11
cereal rye	*Secale cereale*	Guardian	0.20	10
daikon-type radish	*Brassica sativus*	Soil First Select	0.09	11
daikon-type radish	*Brassica sativus*	Tillage	0.00	10
mustard	*Brassica juncea*	Kodiak	0.00	9
oilseed radish	*Brassica sativus*	Image	0.00	10
soybean, SCN susceptible	*Glycine max*	Williams 82	304.81	11

* Data were transformed using the $\log_{10}$ transformation for analysis, but values presented above are untransformed means. Numbers of SCN females on Williams 82 were greater ($P<0.05$) than on any, and all cover crop plants and there were no significant differences in number of females between cover crops.

† Experiments planted in two runs with five replicates in the first run and six replicates in the second run with a total of 11 possible plants. Not all plants survived to the end of the experiment.

‡ Given growth habit of *Lolium multiflorum*, some experimental units had more than one plant.

Figure 1. Root rot severity (%) for plant species evaluated for susceptibility to infection by *Fusarium virguliforme*. Plants were grown in *F. virguliforme*-infested and non-infested soil in greenhouse conditions for 35 days. Data shown is averaged over three separate runs. *Indicates significant difference (*P* < 0.05) between inoculated and non-inoculated plants.

Figure 2. Biomass of plant species evaluated for susceptibility to infection by *Fusarium virguliforme.* Plants were grown in *F. virguliforme*-infested and non-infested soil in greenhouse conditions for 35 days. Data shown is averaged over three separate runs. *Indicates significant difference (*P* < 0.05) between inoculated and non-inoculated plants.

Figure 3. Mean quantity of *Fusarium virguliforme* DNA detected on the roots of inoculated plant species. Plants were grown in *F. virguliforme*-infested soil in greenhouse conditions for 35 days. Letter A indicates run 1 and letter B indicates run 3. *Indicates significant difference (*P* < 0.05) from inoculated soybean.

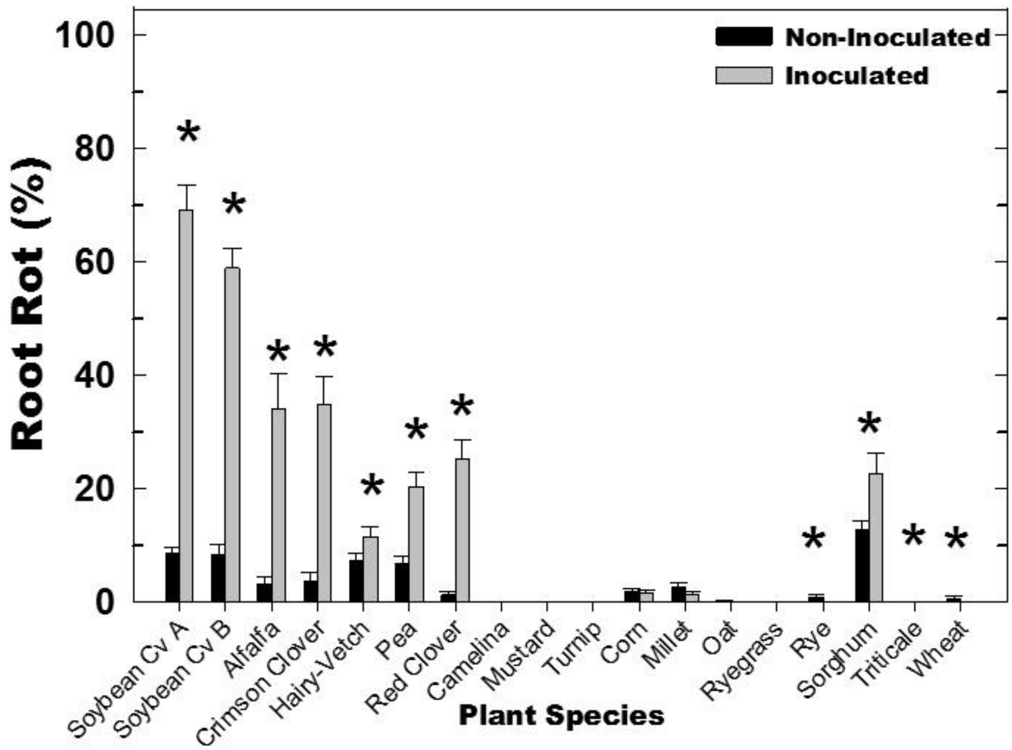
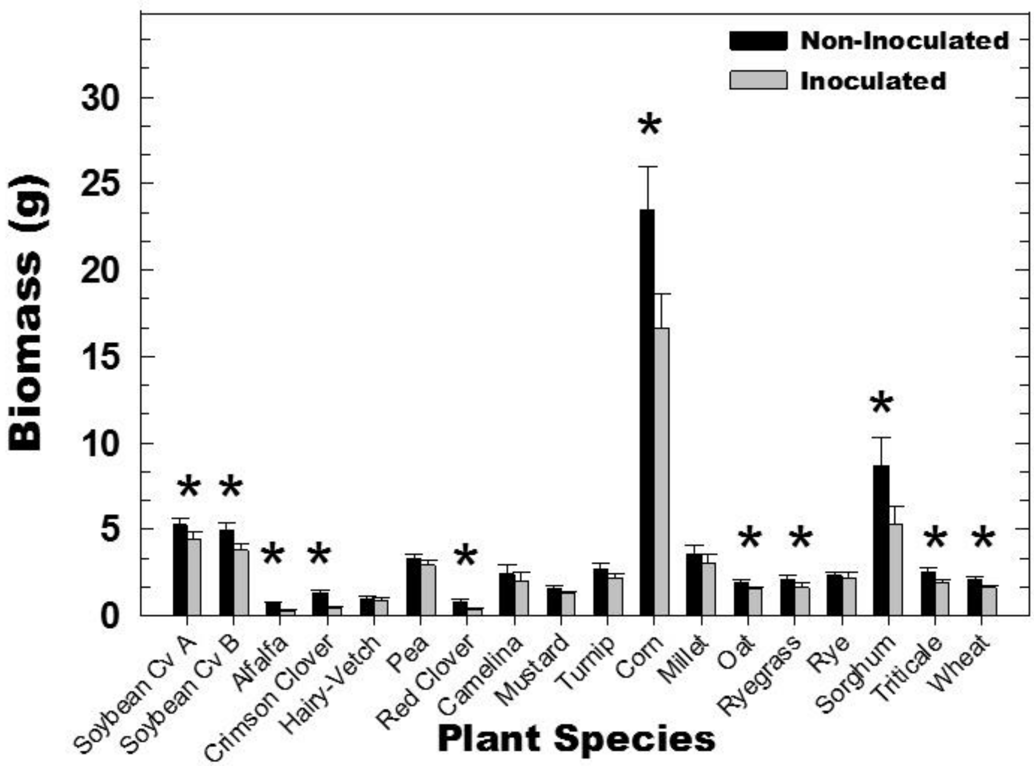
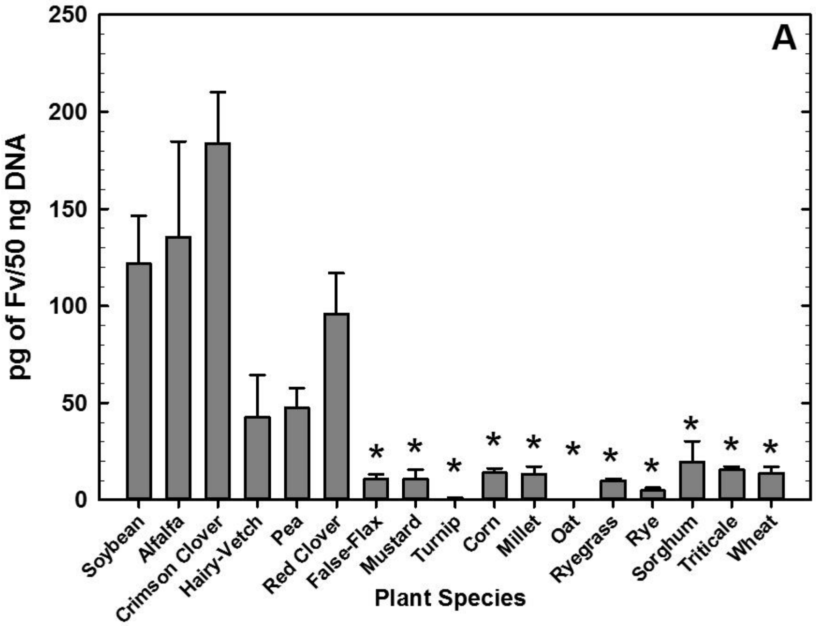

Non-Inoculated
Inoculated
Root Rot (%)
100
80
60
40
20
0
Soybean Cv A
Soybean Cv B
Alfalfa
Crimson Clover
Hairy-Vetch
Pea
Red Clover
Camelina
Mustard
Turnip
Corn
Millet
Oat
Ryegrass
Rye
Sorghum
Triticale
Wheat
Plant Species

Figure 1.



Non-Inoculated
Inoculated
Biomass (g)
0
5
10
15
20
25
30
Soybean Cv A
Soybean Cv B
Alfalfa
Crimson Clover
Hairy-Vetch
Pea
Red Clover
Camelina
Mustard
Turnip
Corn
Millet
Oat
Ryegrass
Rye
Sorghum
Triticale
Wheat
Plant Species

Figure 2.


A
pg of Fv/50 ng DNA
250
200
150
100
50
0
Soybean
Alfalfa
Crimson Clover
Hairy-Vetch
Pea
Red Clover
False-Flax
Mustard
Turnip
Corn
Millet
Oat
Ryegrass
Rye
Sorghum
Triticale
Wheat
Plant Species

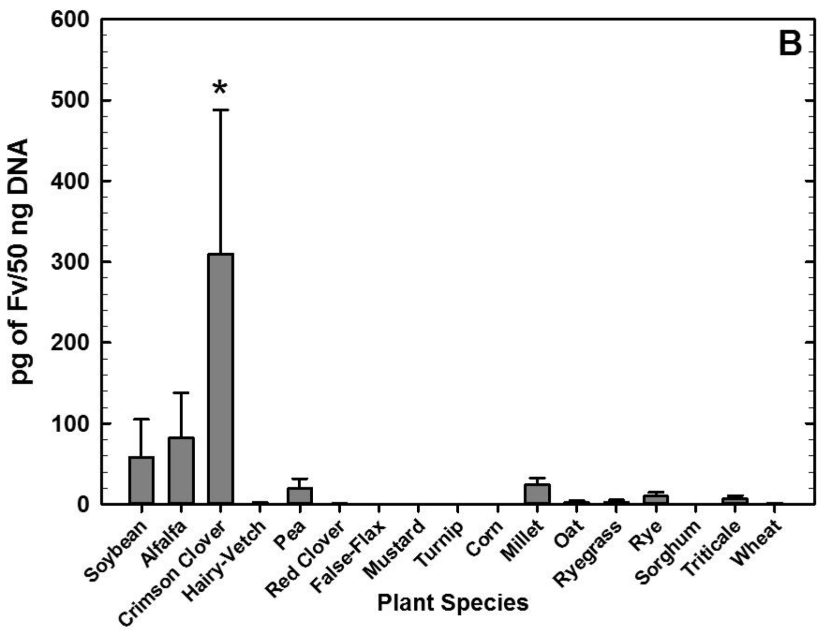

B
pg of Fv/50 ng DNA
600
500
400
300
200
100
0
Soybean
Alfalfa
Crimson Clover
Hairy-Vetch
Pea
Red Clover
False-Flax
Mustard
Turnip
Corn
Millet
Oat
Ryegrass
Rye
Sorghum
Triticale
Wheat
Plant Species

Figure 3.

CHAPTER 3. EFFECTS OF RYE COVER CROP ON SUDDEN DEATH SYNDROME OF SOYBEAN IN FIELD CONDITIONS

Abstract

Soybean sudden death syndrome (SDS), caused by *Fusarium virguliforme,* is an economically important soilborne disease that reduces soybean (*Glycine max*) yield in many regions. Cereal rye (*Secale cereale*) is a winter-hardy cover crop that has been incorporated into the traditional soybean-corn cropping system in Iowa. However, little is known about the effects of this cover crop on SDS. The objective of this study was to evaluate the influence of rye cover crop on SDS. A study was undertaken in 2015 and 2016, in a long-term cover crop trial at Iowa State University's Boyd Farm, located near Boone, Iowa. The trial had plots with a corn (grown as silage) and soybean rotation, with both crops present in each year. This study evaluated SDS development in plots with (1) soybean with no cover crop and (2) soybean planted after termination of a cereal rye cover crop. Plots were arranged in a randomized complete block design with 5 blocks. Rows were inoculated with *F. virguliforme*-infested sorghum at planting. Plant population, plant height, root rot, root and shoot dry weight, SDS foliar incidence and severity, yield, and canopy reflectance were collected from each plot. Overall, cover crop use did not affect plant population, root rot, SDS foliar incidence and severity, or yield. In both years, plants in plots with a rye cover crop were taller, but developed less root mass compared to soybean plants in plots without rye. At the end of the season, plants in the no rye treatment had less green leaf area, as indicated by lower canopy reflectance (%), compared to rye treatments. Based on the conditions of this study, a winter rye cover crop did not affect SDS in the subsequent soybean crop.

Introduction

SDS is a major root rot disease of soybean. Between 2006 and 2009, yield losses due to SDS were estimated in more than 254,000 metric tons in the United States (Koenning and Wrather, 2010). In Iowa, the first report of SDS occurred in 1993 (Yang and Rizvi, 1994). Over the years, the disease has caused several outbreaks (Leandro et al. 2013) and is now considered one of the most important soybean diseases in Iowa.

Even though no single management practice is completely effective at reducing SDS, growers can reduce disease risk by using resistant varieties and seed treatment (Kandel et al. 2016). Seed treatment using the active ingredient fluopyram can reduce disease foliar symptoms by up to 95% (Kandel et al. 2016) and increase yield by up to 25% (Adee, 2015). Although genetic resistance to SDS has improved over the years, no source of complete resistance has been discovered (Zhang et al. 2015). Therefore, there is still a need to find alternative methods to help manage the disease.

Crop rotation is used to control many plant diseases (Lamey and McMullen, 1993; Larkin et al. 2010; Peters et al. 2003). However, this practice has provided inconsistent results for SDS management (Abdelsamad et al. 2012; Rupe et al. 1997; Xing and Westphal, 2009). Rotation of soybean with wheat or sorghum decreased inoculum densities of *F. virguliforme* compared with continuous planting of soybean (Rupe et al. 1997). Abdelsamad et al. (2012) also observed lower levels of SDS in fields with 3-year corn-soybean-oat/red clover and 4-year corn-soybean-oat/alfalfa-alfalfa rotations, as compared to a typical 2-year, soybean-corn rotation. However, the mechanisms behind this suppression are not yet understood because red clover and alfalfa are also hosts to SDS. It is possible, but not demonstrated, that crop diversification benefits microbial community activity, suppresses pathogen populations in soil, and improves soil health.

One potential way to increase crop diversity is to incorporate cover crops into the system. The traditional corn and soybean rotation has been widely used for more than 50 years in the North Central region of United States (Crookston et al. 1991). In recent years, growers in this region have been encouraged to plant winter cover crops, with the goal of reducing erosion and nutrient losses and enhancing soil quality (Kaspar and Singer, 2011; Moore et al. 2014; Pantoja et al. 2016). Rye (*Secale cereale*) has been used as a cover crop for many years (Eckert, 1988; Moschler et al. 1967; Snapp et al. 2005). In Iowa, rye is the most prevalent winter cover crop being planted (Feyereisen et al. 2006; Singer, 2008) because of its winter hardiness and ability to scavenge excess soil nitrate (Kaspar et al. 2012; Rogers and Giddens, 1957).

Long-term use of cover crops may affect soil properties by reducing soil compaction (Chen and Weil, 2010; Williams and Weil, 2004), improving soil structural properties (Villamil et al. 2006), enhancing soil organic matter (Ding et al. 2006; Moore et al. 2014), moderating soil temperature (Wagner-Riddle et al. 1994), and improving microbial communities (Davis et al. 1994). These long-term benefits may affect soilborne pathogens such as *F. virguliforme* and diseases such as SDS by improving soil health. Specifically, compaction increases SDS (Scherm et al. 1998; Vick et al. 2003) and lower soil temperatures favor infection (Gongora-Canul and Leandro, 2011). In addition, improved microbial communities may help provide competition against pathogens (Detheridge et al. 2016; Garbeva et al. 2004; Reddy et al. 2003). According to Ghorbani et al. (2008) enhanced microbial activity can be related to the amount of carbon released in the soil from cover crop residues and can increase competition with pathogen populations. Suppressive soils typically have higher populations of actinomycetes and bacteria, compared to soils conducive to disease (Ghorbani et al. 2008; Ratnadass et al. 2012). Rye cover crops have been demonstrated to enhance soil microbial populations in a soybean production system (Reddy et al. 2003; Wagner et al. 1995). However, changes in soil quality may require several seasons to cause substantial improvements to crop production and health (Kaspar and Singer 2011; Moore et al. 2014).

There is limited information about the impact on soybean SDS when rye cover crops are added to diversify the corn-soybean production system. According to Stone et al. (2004), cover crops can have variable effects on plant diseases. Studies have reported disease reduction (Rothrock et al. 1995; Motisi et al. 2009), disease increase (Hartz et al. 2005), or no effects on disease (Njoroge et al. 2008) in subsequent crops. Therefore, the objective of this study was to determine how the long-term use of a rye cover crop affects soybean SDS in the field.

Material and Methods

Inoculum preparation

Fusarium virguliforme inoculum was produced using sorghum grain as a substrate. Commercial sorghum grain was soaked in tap water for 24 hours, then drained of excess water and placed in mushroom spawn bags. The bags were autoclaved two times for 40 minutes each, on two consecutive days. Ten plugs of *F. virguliforme* isolate NE 305, grown on potato dextrose agar (PDA) plates, were added to the bags (Luckew et al. 2012). The bags were incubated for 4 weeks in the dark at 23ºC, mixing daily to promote a uniform growth of the colonies in the grain. Inoculum was dried on a greenhouse bench for approximately 5 days, and stored at 4ºC until use.

Field experiment

Our field study was conducted at Iowa State University's Boyd Farm, located in Boone County, IA, in the years 2015 and 2016. A corn silage-soybean rotation was established in 2001, where corn silage and soybean were grown alternately in two adjacent fields (Moore et al. 2014). The experiment consisted of two cover crop treatments: (i) rye cover crop after corn silage, followed by soybean; (ii) no cover crop after corn silage, followed by soybean. There were 5 replications for each treatment and each plot was 54.6 m long and 3.8 m wide, with 5 rows spaced 0.76 m apart. The soybean variety used was Pioneer P19T01R and was planted on May 4 and May 6, in 2015 and 2016, respectively. The rye variety Elbon was drilled in a 19 cm row spacing. In 2015 it was terminated on April 30 (4 days prior to soybean planting) and in 2016 on April 25 (11 days prior soybean planting). Herbicide Roundup WeatherMax was used to terminate rye at a concentration of 1.6 L ha^{-1}. Soybeans were inoculated with *F. virguliforme* by applying inoculum in-furrow, at planting, at a rate of 8.2 ml m^{-1}. Inoculum was manually distributed in a 3-m section of each of the three central rows in each plot. Soybean plants were evaluated for stand count, plant height, root rot, root dry weight, SDS incidence, reflectance and yield.

Data collection

Fourteen days after planting (DAP), stand was determined by counting the number of seedlings emerged in a randomly selected 1-m section. Plant height also measured at the same time by arbitrarily assessing 8 plants per row (for a total of 24 plants/plot). This procedure was conducted by measuring plants from the soil line to the tip of the stem at the growing point. For root assessments, plants were sampled at 30 DAP. In each row, 2 plants were randomly collected at 2 different points in each of the 3 central rows. Using a shovel, plants were gently dug from the soil approximately 30 cm deep, in order to preserve the plant root system. A total of 18 plants per plot were sampled in 2015, and 12 plants per plot were sampled in 2016. Roots were carefully washed in tap water and assessed visually as the percentage of total root area exhibiting discoloration to determine root rot levels. Next, samples were oven dried for 48 hours at 70ºC and weighed for root dry weight.

Foliar symptoms of SDS were assessed at the R6 growth stage. Foliar disease incidence (DI) was estimated by counting the number of symptomatic plants in each plot. Foliar disease severity (DS) was visually estimated using a previously reported 0-9 scale (Kandel et al. 2015; Njiti et al. 1998). Foliar disease index (FDX) was calculated by using the formula FDX= DS x DI/9.

During the cropping season, soybean green leaf area was assessed every 7-10 days by using a hand-held, multispectral radiometer (CROPSCAN, Inc., Rochester, MN) to measure the percentage of sunlight reflected from the canopy. Radiometer assessments were conducted in days without clouds and between the hours of 1100 and 1500 CST (Guan and Nutter, 2001). The percentage of sunlight reflected from each plot was measured in the near-infrared region (810 nm) by positioning the radiometer unit 2 m above the soil line; measurements captured reflectance from 1-m-diameter areas of the plant canopy. Two assessments, one entering and one exiting the inoculated area, were obtained per plot.

The center three rows were mechanically harvested using a small plot combine for grain yield on 28 September 2015 (147 DAP) and 27 September 2016 (144 DAP), and grain yield was adjusted to 13% moisture.

Weather

Temperature and precipitation data were collected from weather station IA0200 (42º03’ N, 93º 80’ W) in Ames, IA, located at the ISU Agronomy Farm, approximately 2 km from the field site.

Statistical analysis

Analysis of variance was performed using the PROC MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC) to test differences between the rye cover crop and no cover crop treatments for each of the variables measured: stand count, plant height, root rot ratings, root dry weight, percentage of root rot and yield. Differences in treatments were tested at the P = 0.05 level.

Results

Weather

In May, the mean temperature was 16.6ºC in 2015 and 14.8ºC in 2016, very similar to the 30-year average (16.6ºC) (table 1). For the remainder of the season, temperature in both years was very close to the historic average. One the other hand, there was more rain in 2015 compared to 2016, especially in the early season. June 2016 was very dry (34.1mm) compared to 2015 (175.1 mm) and the 30-year average (123.0 mm) (table 1).

Stand count

Soybean stand was not affected by the rye cover crop in 2015, but greater stands were seen in no-rye plots in 2016 (figure 1A). In 2015, both treatments had similar mean stand counts.

No rye plots had a mean of 31.25 plants m^{-1} (411,184 plants ha^{-1}) while rye treatments had 32.2 plants $meter^{-1}$ (423,684 plants ha^{-1}) (P = 0.6425). In adjacent non-inoculated plots, mean stand counts were 403,907 plants ha^{-1} in the no rye treatment and 407,990 plants ha^{-1} in the rye plots (P = 0.93). In the 2016 season, the mean stand of soybean was 27.2 plants $meter^{-1}$ (357,894 plants ha^{-1}) plants in no rye treatment and 21.2 plants $meter^{-1}$ (278,947 plants ha^{-1}) plants in the rye treatment (P = 0.0327). In the non-inoculated plots, the same pattern was observed; the mean population was 385,243 plants ha^{-1} in the no rye treatments and 346,165 plants ha^{-1} in the rye treatment (P = 0.03).

Plant height

The use of a rye cover crop resulted in taller plant in both years of the study (figure 1B). In 2015, soybean plant height was significantly lower (6.25 cm) in the no rye treatment compared to the rye treatment (6.81 cm) (P = 0.0249). The same pattern was observed in 2016, when plant height was 3.64 cm in the no rye treatments and 4.69 cm in the rye treatment (P = 0.0049).

Root rot

In 2015, root rot severity on individual plants ranged from 0 to 60% at 30 DAP, and did not differ between treatments. Mean root rot severity was 10% in the no rye treatment and 11% in the rye treatment (P = 0.5645) (figure 2B). Approximately 43% of the roots sampled showed root rot greater than 5% (data not shown). In 2016, root rot severity was lower, ranging from 0 to 25% (figure 2B). Despite the low levels of disease in 2016, a significant difference was observed (*P* = 0.0055) between treatments, with root rot being 8.6% in the no rye treatment and 5.7% in the rye treatment.

Dry weight of roots and shoots

In both years, plants in plots without a rye cover crop had significantly greater root dry weight mass compared to plants in plots with rye (figure 2C). In 2015, plants in the no rye treatment had significantly more root dry weight mass (0.84 g) compared to plants in the rye treatment (0.78 g) ($P = 0.0574$). The same results were also observed in 2016, where plants in no rye plots had an average root dry weight of 1.29 g as compared to 0.76 g in plots with rye ($P < 0.0001$). In both years, shoot weight in the rye treatments were lower than in the no rye treatments. Mean shoot weight was 0.67 g in the no rye treatment and 0.61 g in the rye treatments ($P = 0.0381$) in 2015, and 1.05 g in no rye and 0.65g in rye treatments ($P < 0.0001$) in 2016.

SDS severity and incidence

SDS was first observed on 21 August 21 2015 (109 DAP) and on 17 August 17 2016 (103 DAP). SDS incidence was low in both years, ranging from 1 to 15% in 2015, and 1 to 7% in 2016 (figure 2A). The presence or absence of a rye cover crop did not affect SDS incidence in either year ($P = 0.2965$ and $P = 0.1724$ in 2015 and 2016, respectively). The severity of SDS was low in both treatments in both years. In 2015, DS in individual plots ranged 0.3 to 1.0 and in 2016, from 0 to 2.0. No significant difference was detected in either year ($P = 0.2134$ and $P = 1.0$, respectively).

No significant difference was observed in FDX in 2015 and 2016 (P = 0.2262 and P = 0.6416, respectively). The FDX scores for no rye plots ranged from 0 to 1.0 in 2015 and 0 to 1.1 in 2016. Foliar DX scores in plots with rye were also low, ranging from 0.03 to 1.7 in 2015 and 0 to 1.3 in 2016.

Radiometry

In 2015, the crop canopy reflectance was measured 3 times at the end of the season. At 112 and 121 DAP, there were no differences in crop reflectance between the rye and no rye treatments. However, at 128 DAP, plots in the rye treatment showed a significantly higher mean reflectance (22.3%) compared to plots in the no rye treatment (20.0%) (P = 0.0395) (Figure 3). In non-inoculated treatments, reflectance was very similar between no rye and rye treatments throughout the season, and significant differences were only observed at the end of the season. At 121 DAP, the no rye treatment showed lower percentage of reflectance (36.39%) compared to rye treatments (43.1%).

In 2016, the no rye treatment had significantly higher reflectance at 49 (32.1%), 69 (48.1%), and 76 (54.04%) DAP, compared to the rye treatment. However, at 108 DAP, the rye treatment had higher reflectance (31.0%) than no rye (24.7%) (Figure 2). In the non-inoculated plots, reflectance was higher compared to the inoculated plots. For example, at 108 DAP no rye treatments showed 39% of reflectance and rye treatments showed 41% (data not shown).

Yield

There was no statistical difference in yield between treatments in either year (Figure 1C). In 2015, the no rye treatment had an average yield of 4,060 kg ha^{-1}, whereas yield was

4,395 kg ha^{-1} in the rye treatment ($P = 0.2724$). In 2016, yield was 3,180 kg ha^{-1} in no rye treatments and 2,883 kg ha^{-1} in rye treatments and no significant difference was observed.(P = 0.2472).

Discussion

In this study, there was no evidence for the effect of a rye cover crop on SDS. The absence of detectable treatment effects may have been because of low SDS levels in spite of inoculation. The low disease levels may be attributed to the fact that the field had no history of SDS, so natural inoculum was absent. Also, environmental conditions were dry in June 2016, which may have also contributed to disease not getting established in that year.

In general, inoculation did not affect plant populations in the treatments, except in 2016. Lower stands in the rye cover crop plots in 2016 may have been due to the plentiful rye residue which most likely reduced solar incidence on soil surface and soil temperature. In 2016, rye plants were approximately 60cm tall at the time of termination. The presence of residue in the rye treatments also might help to explain the fact that early-season plant height was higher in these treatments in both years. It is likely that the presence of standing rye biomass early in the season resulted in stem elongation of the soybean plants.

With regards to root rot and disease incidence, cover crop effects were mostly not significant, probably because the environment was not conducive to SDS infection. It is important to note that the root rot observed at 30 DAP might have been caused by other soybean pathogens that are present in the soil. This study was conducted in a field with no history of SDS and it would be interesting to test this effect in other locations with higher SDS disease pressure.

Root and shoot dry weight were consistently higher in no rye treatments across years, which again may have been caused by the standing rye residues.

The canopy reflectance data consistently showed lower percent- reflectance in the no rye treatments in both years. This result indicates that there was less green leaf area in the no-rye plots, which agrees with our visual observations late in the season of soybean plants planted into rye staying green longer than the plants in the no rye treatment in both years. However, this effect cannot be attributed to SDS, because SDS disease incidence was low. With regards to yield, there were no differences between treatments. Our study agrees with previous studies which show that a rye cover crop does not impact subsequent soybean yield (Acuña and Villamil, 2014; Gailans and Juchems, 2015).

Thus far, there are only a few studies addressing the effect of cover crops on soilborne diseases of soybean. In a field study, Wen et al. (2012) found lower levels of *Rhizoctonia solani* in soybeans preceded by fallow soil, as compared to soybeans preceded by a rye cover crop, but no effect on SDS was detected in this same study. In a recent study testing the effect of organic soybean transition on disease, Marzano et al. (2015) also did not find significant differences in SDS.

Although rye is not considered to be a host of *F. virguliforme*, it is not clear if the fungus is able to survive in rye residue. Navi and Yang (2016) have reported that *F. virguliforme* can use corn kernels can as a source survival. A recent study showed that corn seedlings following rye cover crop had have higher pathogen densities, such as *F. graminearum, F. oxysporum, P. sylvaticum,* and *P. torulosum*, compared to winter fallow (Bakker et al. 2016). Increased disease in corn following rye may be due to the fact that both plant species are grasses, and are attacked by many of the same pathogens. However, future work is needed to investigate if *F. virguliforme* can colonize and survive on rye residue.

Our goal for this study was to test if rye cover cropping has an effect on SDS. Based on the conditions of this study, there is no evidence that rye can either increase or decrease SDS on soybeans. Interest in cover crops is likely to increase in future years, and more work on this topic is needed to clarify the effects of rye cover crops on soybean SDS.

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Table 1. Mean monthly air temperature and total monthly precipitation near the Boyd Farm field trial, in Ames, IA (2015 and 2016)

Year	Mean monthly temperature (ºC)					
	May	**June**	**July**	**August**	**September**	
2015	16.6	21.5	22.6	20.9	20.8	
2016	14.8	22.8	22.5	22.1	19.5	
30 year average	16.6	21.5	23.3	22	18.2	
	Precipitation (mm)					**Total**
2015	114	175	151	208	128	778
2016	112	34	200	104	188	640
30 year average	120	123	117	123	83	566

Figure 1. (A) Average number of plants in 1 m sections in the no rye and rye treatments. (B) Average plant height at 30 DAP in no rye and rye treatments. (C) Average yield in rye and no rye treatments. Left: 2015 season; Right: 2016 season.

Figure 2. (A) Number of plants showing SDS foliar symptoms at 109 DAP in 2015 and 103 DAP in 2016. (B) Mean percentage of root rot area per treatment. (C) Mean root dry weight in rye and no rye treatments. Left: 2015 season; Right: 2016 season.

Figure 3. Mean percentage of reflectance at 810 nm during the season. Top: 2015 season; Bottom: 2016 season.

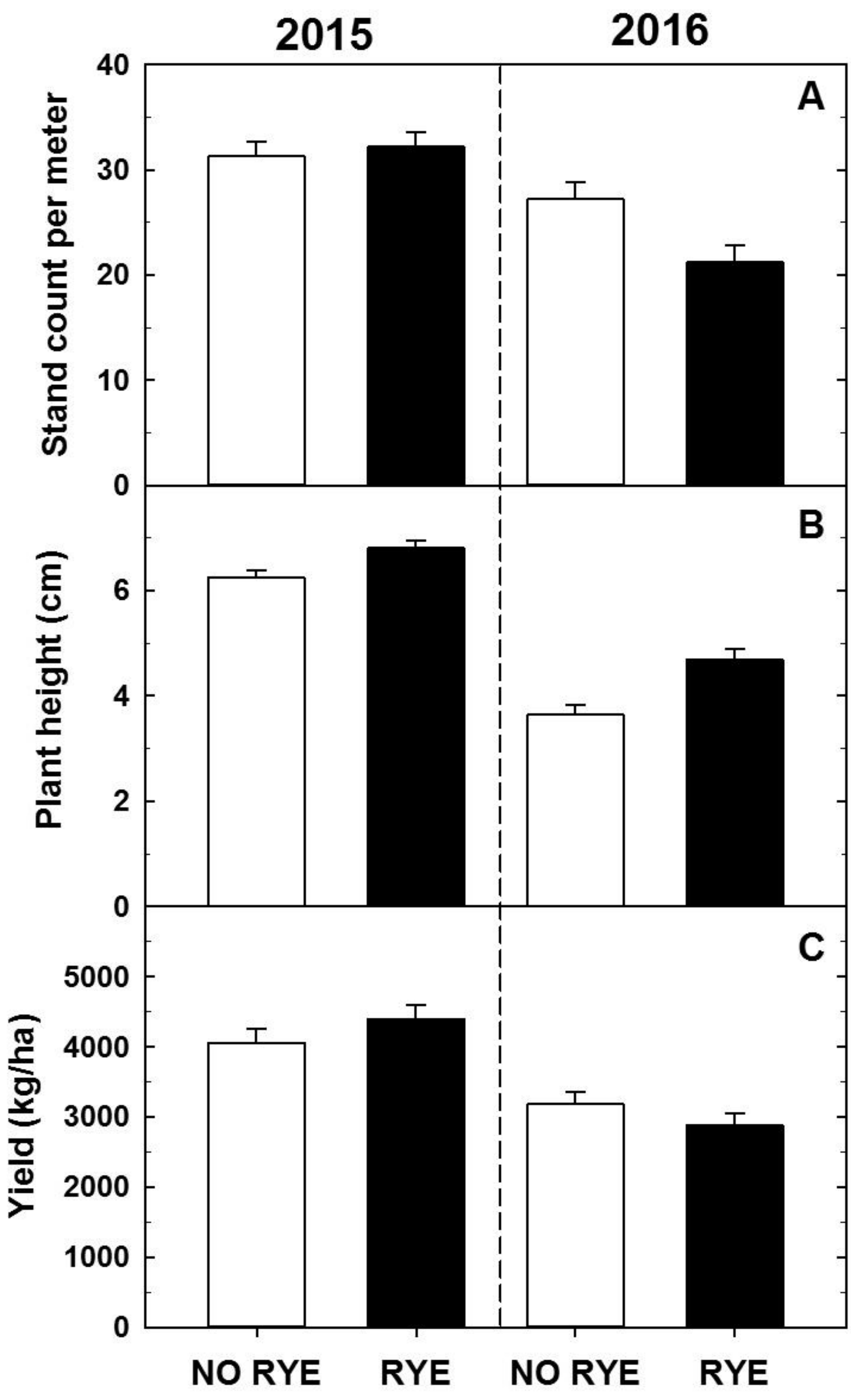
2015
2016
A
B
C
Stand count per meter
Plant height (cm)
Yield (kg/ha)
40
30
20
10
0
6
4
2
0
5000
4000
3000
2000
1000
0
NO RYE
RYE
NO RYE
RYE

Figure 1

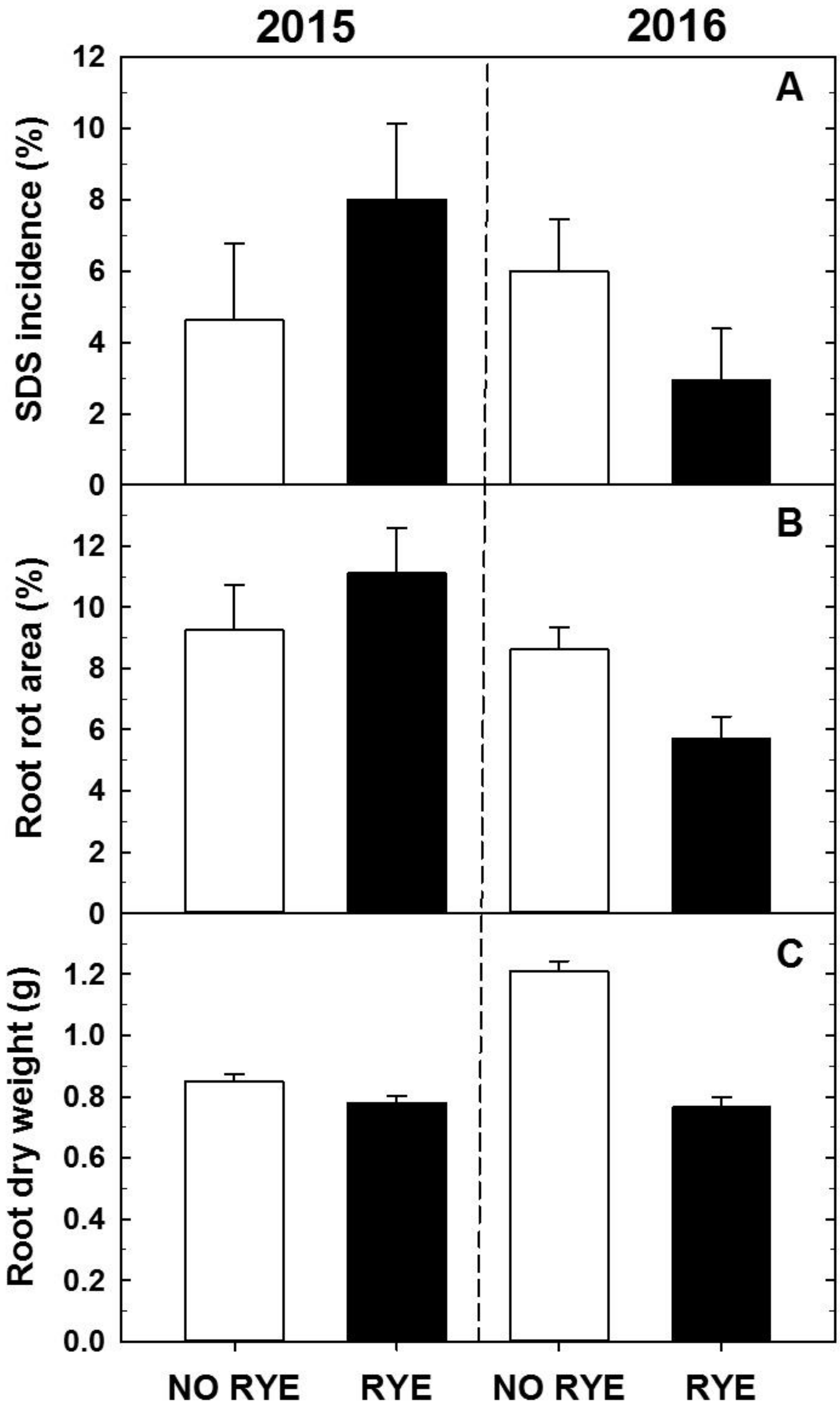
2015
2016
A
B
C
SDS incidence (%)
Root rot area (%)
Root dry weight (g)
0
2
4
6
8
10
12
0.0
0.2
0.4
0.6
0.8
1.0
1.2
NO RYE
RYE
NO RYE
RYE

Figure 2

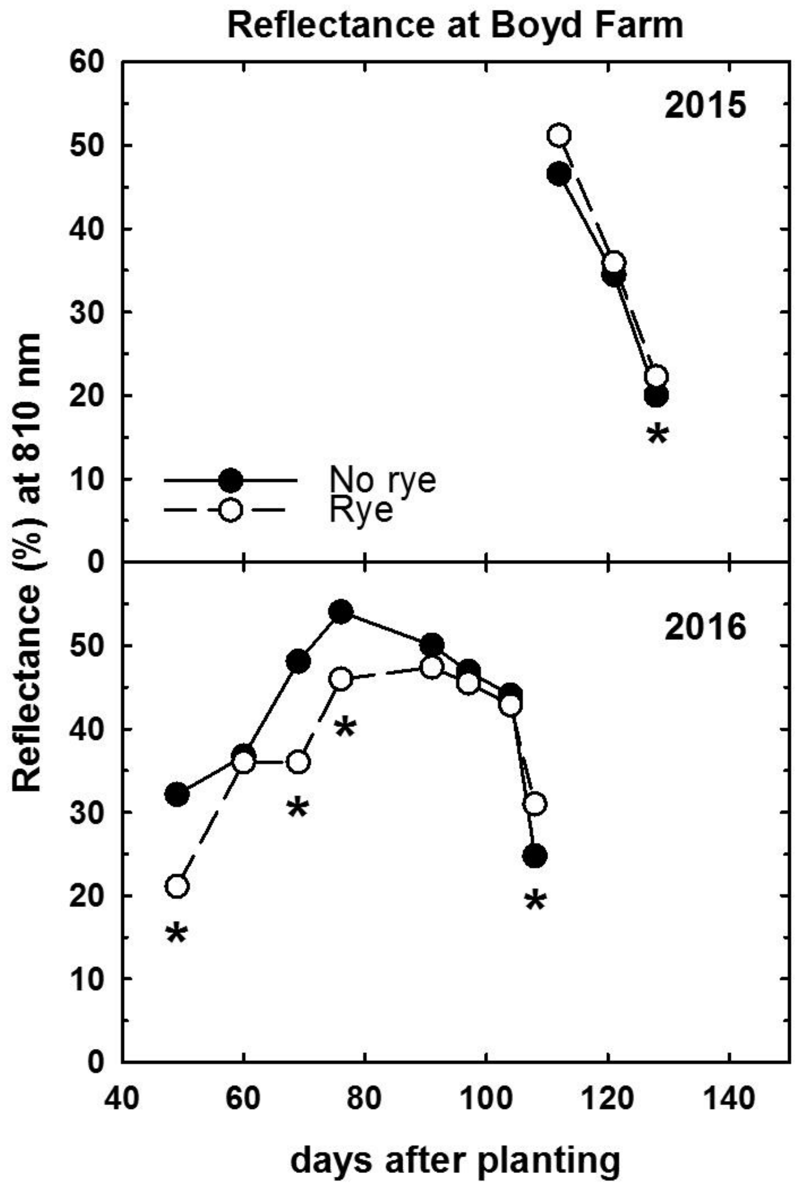
Reflectance at Boyd Farm
2015
2016
No rye
Rye
Reflectance (%) at 810 nm
days after planting
0
10
20
30
40
50
60
40
60
80
100
120
140
*

Figure 3

CHAPTER 4. GENERAL CONCLUSIONS

Based on the findings of this study, we were able to conclude that the legumes species tested for susceptibility of *Fusarium virguliforme* were considered as hosts to the pathogen and they might help to build up inoculum when soybean is not present. On the other hand, *Brassicas* spp. and grasses tested here did not seem to be hosts of *F. virguliforme*. Therefore, they should be considered a preferred option for cover crop for fields with a history of SDS. More work is needed to elucidate if *F. virguliforme* is able to survive asymptomatically in non-host plants or on their residues

The effect of rye cover crop on SDS was investigated in a field trial in two years. Based on the conditions of this study, rye did not seem to affect SDS development in the subsequent soybean crop. However, future research is needed to understand if *F. virguliforme* is able to survive and reproduce on rye residue.

The research findings presented in this study will be useful to researchers and growers to better understand the effect of cover crops on SDS.